

Supplementary

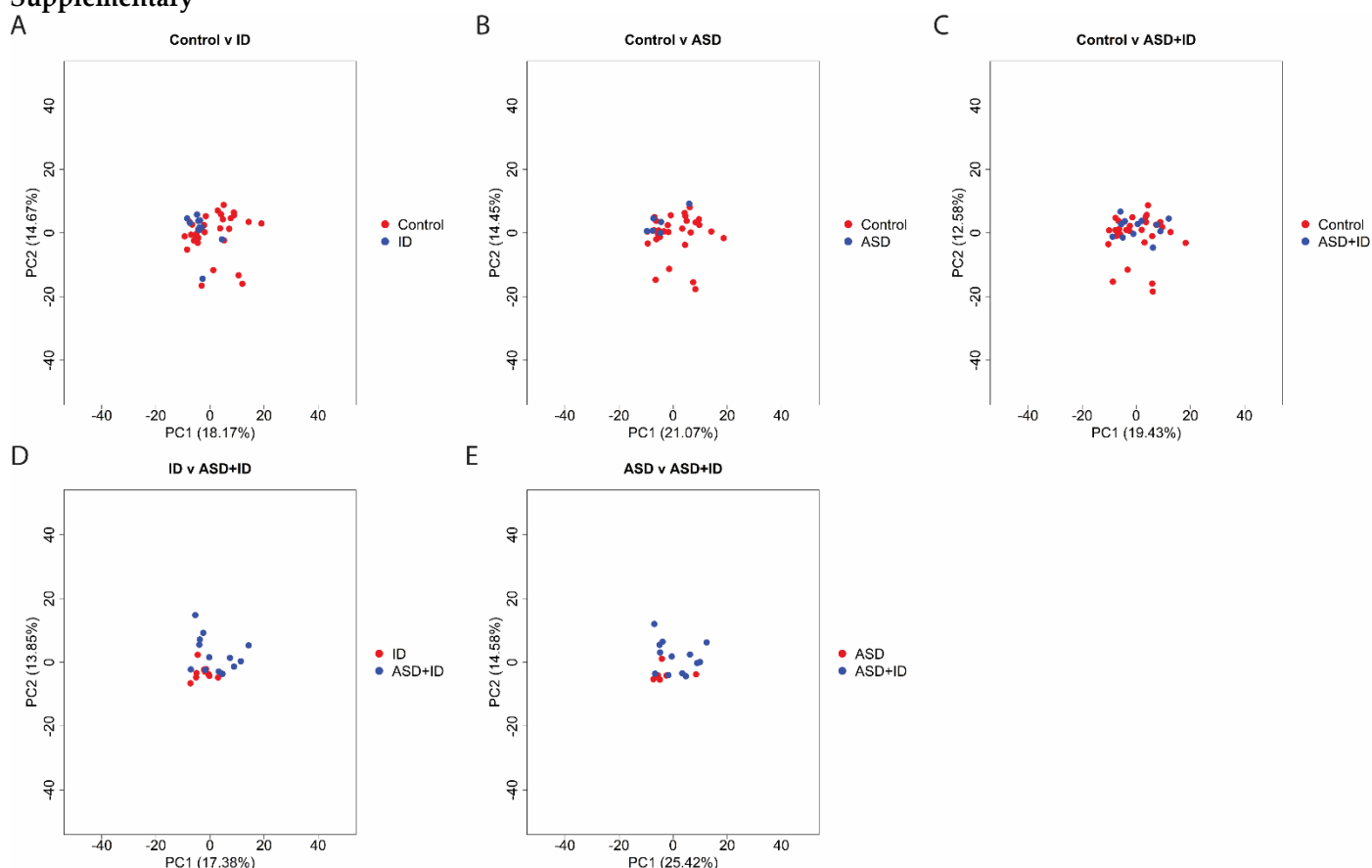


Figure S1. Based on serum miRNAs transcription profile, the PCA analysis revealed no identifiable pattern of the different TANDs in any of the comparisons. A. Controls (TSC without TANDs) and Intellectual Disability (ID). B. Controls (TSC without TANDs) and Autism Spectrum Disorder (ASD). C. Controls (TSC without TANDs) and ASD+ID. D. ID and ASD+ID. E. ASD and ASD+ID. X-axis: Principal Component 1 (PC1). Y-axis: Principal Component 2 (PC2).

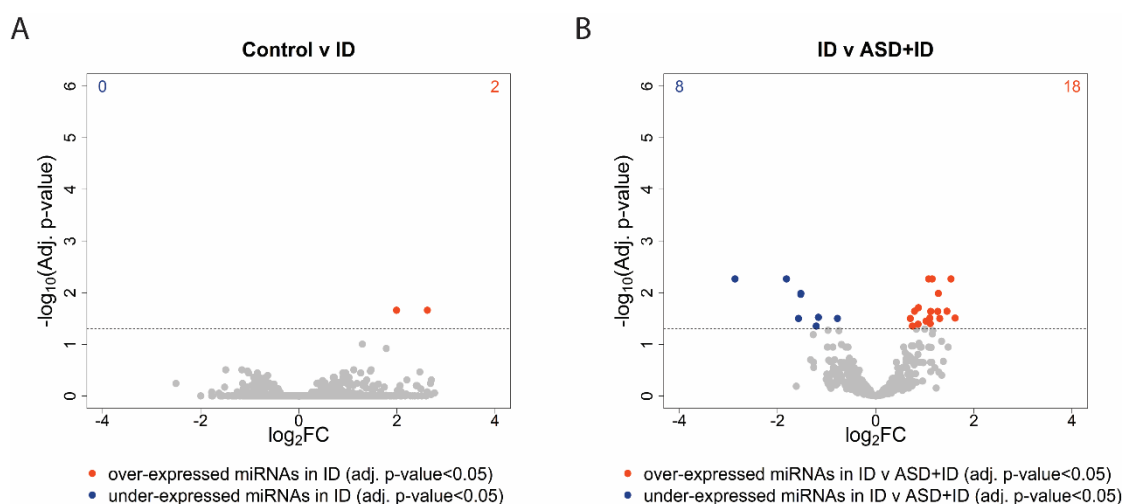


Figure S2. Volcano plot showing the differentially expressed miRNAs (adjusted p-value<0.05). A. Comparing controls (TSC without TANDs) and Intellectual Disability (ID), a total of 2 overexpressed (red) miRNAs were found, and no miRNAs were underexpressed (blue). B. Comparing ID and ASD+ID, a total of 18 overexpressed (red) and 8 underexpressed (blue) miRNAs were found. x-axis: miRNAs expression levels in log2FoldChange (log2FC) scale; y-axis: significance levels in $-\log_{10}(\text{Adj. p-value})$ scale.

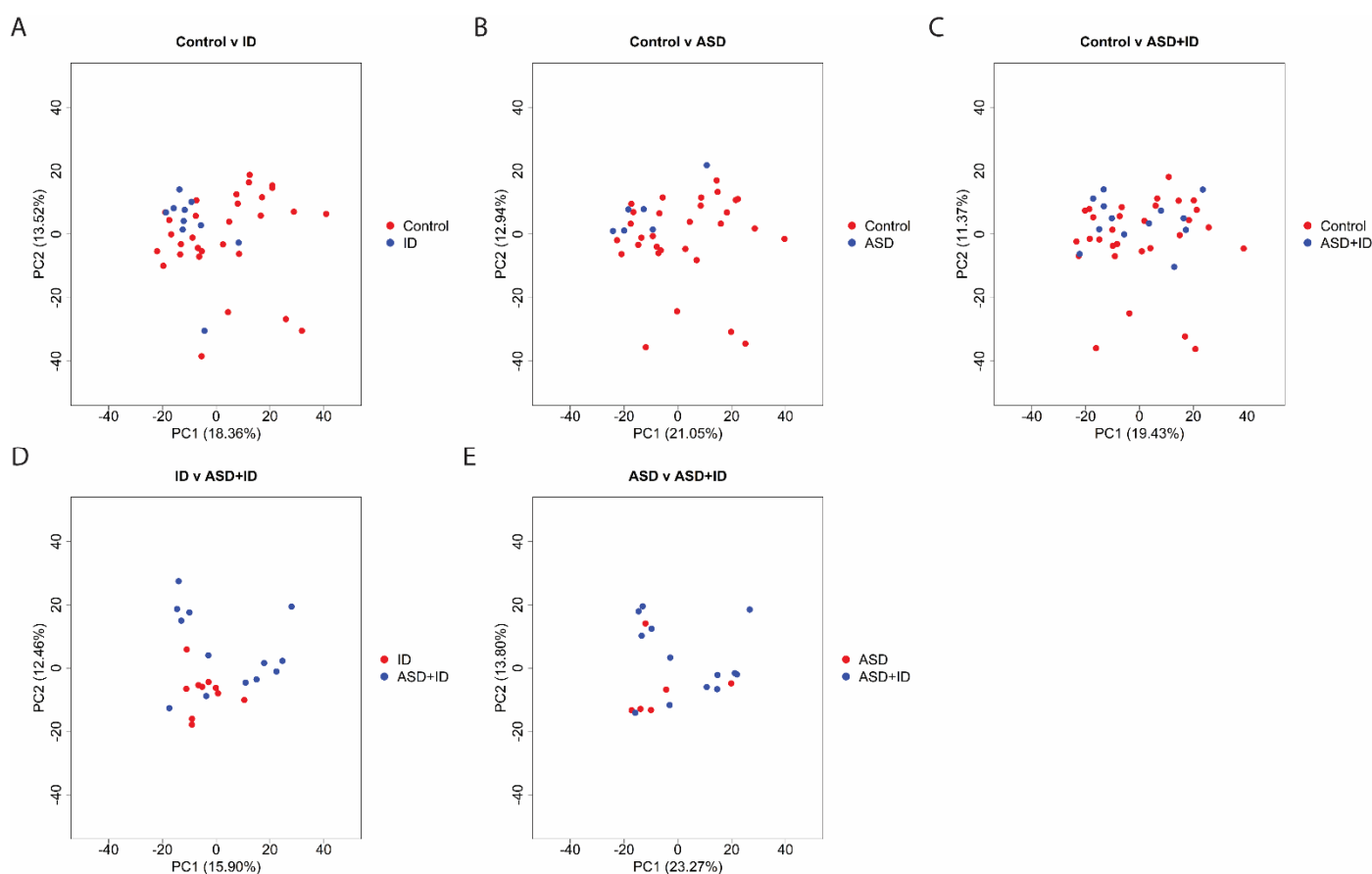


Figure S3. Based on serum isomiRs transcription profile, the PCA analysis revealed no identifiable pattern of the different TANDs in any of the comparisons. A. Controls (TSC without TANDs) and Intellectual Disability (ID). B. Controls (TSC without TANDs) and Autism Spectrum Disorder (ASD). C. Controls (TSC without TANDs) and ASD+ID. D. ID and ASD+ID. E. ASD and ASD+ID. X-axis: Principal Component 1 (PC1). Y-axis: Principal Component 2 (PC2).

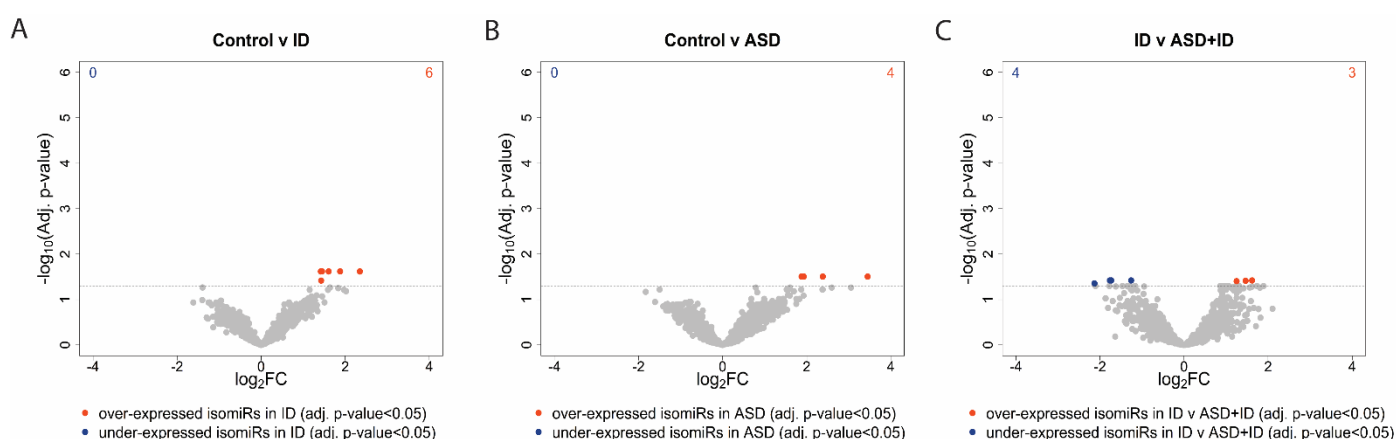


Figure S4. Volcano plot showing the differentially expressed isomiRs (adjusted p-value<0.05). A. Comparing controls (TSC without TANDs) and Intellectual Disability (ID), a total of 6 overexpressed (red) isomiRs were found, and no isomiRs were underexpressed (blue). B. Comparing controls and Autism Spectrum Disorder (ASD), a total of 4 overexpressed (red) and no underexpressed (blue) isomiRs were found. C. Comparing ID and ASD+ID, a total of 3 overexpressed (red) and 4 underexpressed (blue) isomiRs were found. x-axis: isomiRs expression levels in log2FoldChange (log2FC) scale; y-axis: significance levels in $-\log_{10}(\text{Adj. p-value})$ scale.

Table S1: Clinical information of Controls (TSC without TANDs), Intellectual Disability (ID), Autism Spectrum Disorder (ASD) and ASD+ID cases at timepoint V1.

ID	PA Diagnosis	Sex	Age (days)	Used for	TSC mutation	Seizures
1	Control	Male	35	RNA-seq	TSC2	No
2	Control	Male	12	RNA-seq	TSC2	Yes
3	Control	Male	109	RNA-seq	TSC2	Yes
4	Control	Female	26	RNA-seq RT-qPCR	TSC2	No
5	Control	Male	33	RNA-seq	TSC2	Yes
6	Control	Female	42	RNA-seq RT-qPCR	TSC1	No
7	Control	Female	33	RNA-seq RT-qPCR	TSC2	No
8	Control	Male	33	RNA-seq	TSC2	Yes
9	Control	Female	49	RNA-seq RT-qPCR	TSC2	No
10	Control	Male	32	RNA-seq RT-qPCR	TSC1	No
11	Control	Female	39	RNA-seq RT-qPCR	TSC2	Yes
12	Control	Male	13	RNA-seq	TSC1	No
13	Control	Male	39	RNA-seq RT-qPCR	TSC2	Yes
14	Control	Male	27	RNA-seq RT-qPCR	TSC2	Yes

15	Control	Male	53	RNA-seq	TSC2	Yes
16	Control	Female	27	RNA-seq	TSC2	Yes
17	Control	Male	117	RNA-seq	TSC1	No
18	Control	Male	15	RNA-seq	NMI	No
19	Control	Female	15	RNA-seq	TSC2	Yes
20	Control	Female	81	RNA-seq	TSC2	No
21	Control	Male	68	RNA-seq	TSC2	Yes
22	Control	Female	28	RNA-seq	TSC2	Yes
23	Control	Female	35	RNA-seq	TSC2	No
24	Control	Female	117	RNA-seq	TSC2	Yes
25	Control	Male	41	RNA-seq	TSC1	Yes
26	Control	Female	88	RNA-seq	TSC2	Yes
27	Control	Female	56	RNA-seq	TSC2	Yes
28	Control	Female	111	RNA-seq	TSC1	No
29	Control	Female	39	RNA-seq	TSC2	No
30	Control	Male	0	RNA-seq	TSC1	Yes
31	ID	Female	32	RNA-seq RT-qPCR	TSC2	Yes
32	ID	Female	18	RNA-seq RT-qPCR	TSC2	Yes

33	ID	Female	34	RNA-seq RT-qPCR	TSC2	Yes
34	ID	Male	7	RNA-seq RT-qPCR	TSC2	Yes
35	ID	Male	15	RNA-seq RT-qPCR	TSC1	Yes
36	ID	Female	20	RNA-seq RT-qPCR	TSC2	Yes
37	ID	Female	9	RNA-seq RT-qPCR	TSC2	Yes
38	ID	Female	15	RNA-seq RT-qPCR	TSC2	Yes
39	ID	Male	18	RNA-seq	TSC2	Yes
40	ID	Female	34	RNA-seq RT-qPCR	TSC1	Yes
41	ASD	Male	11	RNA-seq	TSC2	Yes
42	ASD	Male	12	RNA-seq	TSC1	Yes
43	ASD	Female	18	RNA-seq	TSC1	No
44	ASD	Male	34	RNA-seq	TSC1	Yes
45	ASD	Male	49	RNA-seq	TSC2	Yes
46	ASD	Male	34	RNA-seq	TSC2	Yes
47	ASD+ID	Male	84	RNA-seq RT-qPCR	TSC1	No
48	ASD+ID	Male	120	RNA-seq	TSC2	Yes

49	ASD+ID	Male	25	RNA-seq RT-qPCR	TSC2	Yes
50	ASD+ID	Male	18	RNA-seq RT-qPCR	TSC2	Yes
51	ASD+ID	Male	71	RNA-seq	TSC2	Yes
52	ASD+ID	Male	120	RNA-seq	TSC2	Yes
53	ASD+ID	Male	15	RNA-seq	TSC2	Yes
54	ASD+ID	Female	19	RNA-seq RT-qPCR	TSC2	Yes
55	ASD+ID	Female	68	RNA-seq RT-qPCR	TSC2	Yes
56	ASD+ID	Male	6	RNA-seq	TSC2	Yes
57	ASD+ID	Male	49	RNA-seq RT-qPCR	TSC2	Yes
58	ASD+ID	Female	8	RNA-seq	TSC2	Yes
59	ASD+ID	Female	102	RNA-seq RT-qPCR	TSC2	Yes

Abbreviations: RNA-seq, RNA sequencing; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

Table S2: miRNAs ROC analysis. Chosen reference gene for each comparison.

Comparison	Reference gene
Controls v ID	hsa-miR-26b-3p
Controls v ASD	hsa-miR-26b-3p
Controls v ASD+ID	hsa-miR-26b-3p
ID v ASD+ID	hsa-miR-26b-3p
ASD v ASD+ID	hsa-miR-1307-5p

Table S3: isomiRs ROC analysis. Chosen reference gene for each comparison.

Comparison	Reference gene complete sequence
Controls v ID	hsa-miR-378a-3p_miRNA_ACTGGACTTGGAGTCAGAAGGC
Controls v ASD	hsa-miR-769-5p_miRNA_TGAGACCTCTGGGTTCTGAGCT
Controls v ASD+ID	hsa-miR-423-3p_trim1_AGCTCGGTCTGAGGCCCTCAG
ID v ASD+ID	hsa-miR-3184-3p_A_5prime_AAAAGTCTCGCTCTTGCCCTCA
ASD v ASD+ID	hsa-miR-3184-3p_A_5prime_AAAAGTCTCGCTCTTGCCCTCA

Table S4: Top 10 miRNA biomarkers for Intellectual Disability (ID), Autism Spectrum Disorder (ASD), ASD+ID, ID v ASD+ID and ASD v ASD+ID.

	Comparison	miRNA	AUC
1	Control v ID	hsa-miR-409-5p*	0.8800000
2	Control v ID	hsa-miR-494-3p	0.8200000
3	Control v ID	hsa-miR-1301-3p*	0.8166667
4	Control v ID	hsa-miR-323a-3p	0.8100000
5	Control v ID	hsa-miR-485-3p	0.8100000
6	Control v ID	hsa-miR-183-5p	0.8033333
7	Control v ID	hsa-miR-487b-3p	0.8033333
8	Control v ID	hsa-let-7i-3p	0.7966667
9	Control v ID	hsa-miR-145-5p*	0.7966667
10	Control v ID	hsa-miR-382-5p	0.7866667
1	Control v ASD	hsa-let-7i-3p	0.8944444
2	Control v ASD	hsa-miR-345-5p	0.8888889
3	Control v ASD	hsa-miR-192-5p	0.8833333
4	Control v ASD	hsa-miR-103a-3p	0.8777778
5	Control v ASD	hsa-miR-181a-5p	0.8722222
6	Control v ASD	hsa-miR-130a-3p	0.8611111
7	Control v ASD	hsa-miR-182-5p	0.8555556
8	Control v ASD	hsa-miR-378a-3p	0.8555556
9	Control v ASD	hsa-miR-183-5p	0.8500000
10	Control v ASD	hsa-miR-96-5p	0.8500000
1	Control v ASD+ID	hsa-miR-214-5p	0.8166667
2	Control v ASD+ID	hsa-miR-550b-2-5p	0.7282051
3	Control v ASD+ID	hsa-miR-374a-5p	0.7230769
4	Control v ASD+ID	hsa-miR-4685-3p	0.7230769
5	Control v ASD+ID	hsa-miR-320b	0.7179487

6	Control v ASD+ID	hsa-miR-376c-3p	0.7141026
7	Control v ASD+ID	hsa-miR-199b-5p	0.7038462
8	Control v ASD+ID	hsa-miR-376a-5p	0.7012821
9	Control v ASD+ID	hsa-miR-299-3p	0.7012821
10	Control v ASD+ID	hsa-miR-450b-5p	0.6974359
1	ID v ASD+ID	hsa-miR-494-3p	0.9076923
2	ID v ASD+ID	hsa-let-7i-3p	0.8923077
3	ID v ASD+ID	hsa-miR-409-5p	0.8692308
4	ID v ASD+ID	hsa-miR-323a-3p	0.8615385
5	ID v ASD+ID	hsa-miR-369-3p	0.8538462
6	ID v ASD+ID	hsa-miR-589-5p	0.8538462
7	ID v ASD+ID	hsa-miR-182-5p	0.8384615
8	ID v ASD+ID	hsa-miR-183-5p	0.8384615
9	ID v ASD+ID	hsa-miR-7706	0.8384615
10	ID v ASD+ID	hsa-miR-320b	0.8307692
1	ASD v ASD+ID	hsa-miR-103a-3p*	0.9230769
2	ASD v ASD+ID	hsa-miR-487b-3p	0.9102564
3	ASD v ASD+ID	hsa-miR-485-3p	0.8974359
4	ASD v ASD+ID	hsa-miR-625-3p	0.8846154
5	ASD v ASD+ID	hsa-miR-150-3p	0.8589744
6	ASD v ASD+ID	hsa-miR-16-5p	0.8333333
7	ASD v ASD+ID	hsa-miR-22-3p	0.8333333
8	ASD v ASD+ID	hsa-miR-652-3p	0.8333333
9	ASD v ASD+ID	hsa-miR-25-3p	0.8076923
10	ASD v ASD+ID	hsa-miR-589-5p	0.8076923

* Indicates miRNAs present in miRNA biomarker panels

Table S5: ROC curve permutation analysis. Listed are the AUC for the miRNA biomarker in that comparison, the number of permutations run in the analysis and the p-value. p-value < 0.05 was considered significant.

Comparison	AUC of biomarker	Permutation number	Permutation p-value
Controls v ID	0.88	30000	0.000033
Controls v ASD	0.894	30000	0.001267
Controls v ASD+ID	0.817	30000	0.000567
ID v ASD+ID	0.908	30000	0.000433
ASD v ASD+ID	0.923	30000	0.0025

Table S6: Top 10 isomiR biomarkers for Intellectual Disability (ID), Autism Spectrum Disorder (ASD), ASD+ID, ID v ASD+ID and ASD v ASD+ID.

	Comparison	miRNA	AUC
1	Control v ID	hsa-miR-409-3p_AT_3prime_GAATGTTGCTCGGTGAACCCCTAT	0.89
2	Control v ID	hsa-miR-411-5p_trim1_TAGTAGACCGTATAGCGTAC	0.856666667
3	Control v ID	hsa-miR-145-5p_T_3prime_GTCCAGTTTCCCAGGAATCCCTT	0.843333333
4	Control v ID	hsa-miR-409-5p_miRNA_AGGTTACCCGAGCAACTTTGCA T	0.843333333
5	Control v ID	hsa-miR-382-5p_miRNA_GAAGTTGTTTCGTGGTGGATTTCG	0.833333333
6	Control v ID	hsa-miR-1301-3p_miRNA_TTGCAGCTGCCTGGGAGTGACTTC	0.83
7	Control v ID	hsa-miR-323a-3p_G_5prime_GCACATTACACGGTCGACCTC T	0.826666667
8	Control v ID	hsa-miR-409-3p_AA_3prime_GAATGTTGCTCGGTGAACCCCTAA	0.826666667
9	Control v ID	hsa-miR-493-5p_trim2_TTGTACATGGTAGGCTTTCA	0.826666667

10	Control v ID	hsa-miR-409- 3p_TT_3prime_GAATGTTGCTCGGTGAACCC CTTT	0.823333333
1	Control v ASD	hsa-miR-28- 3p_G_3prime_CACTAGATTGTGAGCTCCTGG AG	0.922222222
2	Control v ASD	hsa-miR- 3615_GC_3prime_TCTCTCGGCTCCTCGCGGC TCGC	0.905555556
3	Control v ASD	hsa-miR-671- 3p_T_3prime_TCCGGTTCTCAGGGCTCCACC T	0.905555556
4	Control v ASD	hsa-miR-328- 3p_miRNA_CTGGCCCTCTCTGCCCTTCCGT	0.9
5	Control v ASD	hsa-miR-485- 5p_trim1_AGAGGCTGGCCGTGATGAATT	0.9
6	Control v ASD	hsa-miR-92a- 3p_CT_3prime_TATTGCACTTGTCGCCGCCT GTCT	0.9
7	Control v ASD	hsa-miR-485- 3p_miRNA_GTCATACACGGCTCTCCTCTCT	0.894444444
8	Control v ASD	hsa-miR-6842- 3p_T_3prime TTGGCTGGTCTCTGCTCCGCA GT	0.894444444
9	Control v ASD	hsa-miR-99b- 5p_A_3prime_CACCCGTAGAACCGACCTTG CGA	0.888888889
10	Control v ASD	hsa-miR-127- 3p_TTA_3prime_TCGGATCCGTCTGAGCTTG GCTTTA	0.883333333
1	Control v ASD+ID	hsa-miR-199b- 5p_miRNA_CCCAGTGTTTAGACTATCTGTTC	0.787179487
2	Control v ASD+ID	hsa-miR-505- 3p_miRNA_CGTCAACACTTGCTGGTTTCCT	0.785897436
3	Control v ASD+ID	hsa-miR-192- 5p_trim1_CTGACCTATGAATTGACAGC	0.779487179
4	Control v ASD+ID	hsa-miR-140- 3p_AAA_3prime_TACCACAGGGTAGAACCA CGGAAA	0.775641026

5	Control v ASD+ID	hsa-miR-148b- 3p_miRNA_TCAGTGCATCACAGAACTTTGT	0.769230769
6	Control v ASD+ID	hsa-miR-29a- 3p_miRNA_TAGCACCATCTGAAATCGGTTA	0.766666667
7	Control v ASD+ID	hsa-miR-660- 5p_A_3prime_TACCCATTGCATATCGGAGTT GA	0.766666667
8	Control v ASD+ID	hsa-miR-22- 3p_T_3prime_AAGCTGCCAGTTGAAGAACT GTT	0.761538462
9	Control v ASD+ID	hsa-miR-378a- 3p_trim2_ACTGGACTTGGAGTCAGAAG	0.761538462
10	Control v ASD+ID	hsa-miR-3120- 3p_G_5prime_GCACAGCAAGTGTAGACAGG CA	0.758974359
1	ID v ASD+ID	hsa-miR-323a- 3p_G_5prime_GCACATTACACGGTCGACCTC T	0.969230769
2	ID v ASD+ID	hsa-miR-409- 3p_AT_3prime_GAATGTTGCTCGGTGAACCC CTAT	0.969230769
3	ID v ASD+ID	hsa-miR-409- 5p_miRNA_AGGTTACCCGAGCAACTTTGCA T	0.915384615
4	ID v ASD+ID	hsa-miR-432- 5p_trim2_TCTTGGAGTAGGTCATTGGGT	0.907692308
5	ID v ASD+ID	hsa-miR-433- 3p_trim1_ATCATGATGGGCTCCTCGGTG	0.892307692
6	ID v ASD+ID	hsa-miR-485- 3p_miRNA_GTCATACACGGCTCTCCTCTCT	0.892307692
7	ID v ASD+ID	hsa-miR-136- 3p_miRNA_CATCATCGTCTCAAATGAGTCT	0.884615385
8	ID v ASD+ID	hsa-miR-381- 3p_trim1_TATACAAGGGCAAGCTCTCTG	0.884615385
9	ID v ASD+ID	hsa-miR-487b- 3p_miRNA_AATCGTACAGGGTCATCCACTT	0.884615385
10	ID v ASD+ID	hsa-miR-127- 3p_TT_3prime_TCGGATCCGTCTGAGCTTGG CTTT	0.876923077

1	ASD v ASD+ID	hsa-miR-221- 3p_trim3_AGCTACATTGTCTGCTGGGT	0.974358974
2	ASD v ASD+ID	hsa-miR-221- 3p_trim2_AGCTACATTGTCTGCTGGGTT	0.961538462
3	ASD v ASD+ID	hsa-miR-182- 5p_trim4_TTTGGCAATGGTAGAACTCA	0.948717949
4	ASD v ASD+ID	hsa-miR-423- 3p_trim5_AGCTCGGTCTGAGGCCCC	0.948717949
5	ASD v ASD+ID	hsa-let-7i- 5p_trim3_TGAGGTAGTAGTTTGCT	0.935897436
6	ASD v ASD+ID	hsa-miR-423- 5p_trim3_TGAGGGGCAGAGAGCGAGAC	0.935897436
7	ASD v ASD+ID	hsa-miR-181a- 3p_trim3_ACCATCGACCGTTGATTGT	0.923076923
8	ASD v ASD+ID	hsa-miR-181a- 5p_trim2_AACATTCAACGCTGTCGGTGA	0.923076923
9	ASD v ASD+ID	hsa-miR-378a- 3p_T_3prime_ACTGGACTTGGAGTCAGAAG GCT	0.923076923
10	ASD v ASD+ID	hsa-miR-181c- 5p_trim1_AACATTCAACCTGTCGGTGAG	0.91025641

Table S7: ROC curve permutation analysis. Listed are the AUC for the isomiR biomarker in that comparison, the number of permutations run in the analysis and the p-value. p-value < 0.05 was considered significant.

Comparison	AUC of biomarker	Permutation number	Permutation p-value
Controls v ID	0.89	30000	0.0001
Controls v ASD	0.922	30000	0.000533
Controls v ASD+ID	0.787	30000	0.002333
ID v ASD+ID	0.969	30000	0 *
ASD v ASD+ID	0.974	30000	0.0003

* No permutations showed higher AUC than our found AUC